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The Effects of pH, Temperature, Light Intensity, Light Quality, and Moisture Levels on Spore Germination in *Cheilanthes feei* of Southeast Missouri

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ABSTRACT.—*Cheilanthes feei* is a xerophytic fern that is broadly distributed throughout the United States west of the Mississippi. Although it has a broad distribution, it occupies a very narrow niche. In southeast Missouri, *C. feei* inhabits crevices of limestone bluffs, in full sun, approximately 0.5–1.0 m from the top of the bluffs. The physiological basis for the fern's restriction to this xeric environment is unclear. In this study, *C. feei* spores were subjected to a broad range of temperatures, pH, and light intensities, to varied light qualities, and to different moisture levels. Results indicate that *C. feei* spores can germinate under a wide variety of conditions. However, data suggest that spore germination optima and optimal conditions for protonemal growth overlap narrowly. The disparity in optimum conditions may be a partial basis for the broad distribution and narrow niche of *C. feei*.

Cheilanthes feei Moore is a common fern that is widely distributed in North America. Its range extends primarily from southwestern Canada, south to north central Mexico, and east to the Mississippi and Ohio River valleys of Midwestern United States (Mickel, 1979). Although *C. feei* is common, it is unusual in several ways. First, *C. feei* is a xerophytic fern. This is somewhat of an oxymoron, since fern gametes are typically free-swimming and most ferns are restricted to moist environments. However, in *C. feei* and many cheilanthoid ferns, the need for water for reproduction is circumvented by apogamy. Secondly, although *C. feei* is widely distributed, it occupies a very narrow niche. In southeast Missouri, *C. feei* typically grows in crevices of limestone bluffs, typically facing south in full sun and approximately 0.5–1.0 m below bluff tops.

The basis for this habitat restriction is unclear. However, there are many feasible explanations. It is possible that the fern cannot compete with more vigorous species in mesophytic habitats, but can survive in more xeric environments. *Cheilanthes feei*, like other *Cheilanthes* species, is characterized by some adaptations that can reduce water loss, such as numerous trichomes and a small surface area to volume ratio (Hevly, 1963; Gratani *et al.*, 1998). This would be analogous to the saguaro cactus, *Cereus giganteus*, which is restricted to areas of intense sunlight, since the thick hydrodermis causes it to be light-limited (Darling, 1989). Another possible explanation for habitat restriction in *Cheilanthes feei* is that the narrow niche that *C. feei* occupies may provide the optimal growth conditions for the fern, so that the incidence of *C. feei* in other habitats is very low. A final possibility is habitat specificity. *Cheilanthes feei* may be restricted to its habitat based on unique characteristics of both the fern and its environment.

This study addresses the physiological basis for the restriction of *Cheilanthes feei* to limestone bluff crevices. Optimal conditions for spore germination are often a reflection of optimal growth conditions for the entire life cycles of the ferns. Since the fern gametophyte is the most vulnerable stage in the fern life cycle, gametophyte physiology and the necessary condition ranges for growth and development are limiting for ferns. Hence, optimal conditions for gametophyte survival and development are typically congruous with optimal spore germination conditions (Raghavan, 1980; notwithstanding necessary changes in light qualities which spur developmental changes). In addition, environmental conditions that negatively affect spore germination typically reflect the physiological limitations of a fern species. An obvious example is the need for water. Most spores require the presence of water and undergo imbibition prior to germination. The gametophyte stage and the fertilization process also require at least a film of water.

Previous studies demonstrate that cheilanthoid ferns, with respect to spore germination requirements, may be physiologically similar to mesophytic ferns. For example, most ferns germinate and develop best at a slightly acidic pH, at 25°C, in moist conditions, under red light (a phytochrome response), and moderate light intensity (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Raghavan, 1980). With respect to pH, temperature, light quality and light intensity, these are also the optimal conditions for spore germination in several cheilanthoid species (Hevly, 1963; Raghavan, 1973). Still, these ferns have very different distributions than *C. feei*, and optimal spore germination conditions for *C. feei* may be different as well. Therefore, we examined the effects of pH, temperature, light intensity, light quality, and moisture levels on germination rates of *C. feei* spores. In addition, we measured the potential water content and porosity of rock substrate in *C. feei* habitat.

Materials and Methods

Plant Collection.—*Cheilanthes feei* sporophylls were collected in the fall and winter of 2000 from Reis Biological Station, Steeleville, MO. To harvest spores, sporophylls were crushed using a mortar and pestle. *Cheilanthes feei* spores average 67.0 μm in diameter (Knobloch, 1969) and spores were separated from the plant material using a 75.0 μm brass mesh sieve and stored at 4° C in the dark.

Culture Conditions.—Although this study addresses optimal spore conditions for *Cheilanthes feei*, there is previously no information available on optimal culture conditions, growth medium contents or osmolality, with the exception of anecdotal information (Siegler, 2002). Therefore, a standard growth medium, Knudson's-C (C-Fern, 2001), that contains salts, iron, phosphate buffer, sugar as an osmoticum, and agar for solidification, was selected. The effects on spore germination of any of these contents, such as sucrose, on *C. feei* are unknown, and have varying effects on germination in other species (Raghavan, 1989; Sheffield *et al.*, 2001). Therefore, we strove only to make them consistent through all of the treatments. All treatments then, were cul-

TABLE 1. Standard conditions within experiments were 25°C, pH 5.5, continuous white at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, identifiably saturated agar-based Knudson's-C. the exceptions were the light quality and light intensity experiments, in which 25°C or 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was difficult to maintain in some light intensities or qualities.

Variable	pH	Temperature	Light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Light quality	Moisture level of substrate
pH	NA	25°C	100	White	Saturated
Temperature	5.5	NA	100	White	Saturated
Light intensity	5.5	33°C	NA	White	Saturated
Light quality	5.5	33°C	75	NA	Saturated
Moisture level of substrate	5.5	25°C	100	White	NA
Dark plus	5.5	25°C	100 (45 min pre-stimulus)	White/dark	Saturated
True dark	5.5	25°C	0.04 (45 min pre-stimulus)	White/dark	Saturated

tured on the same growth medium lot. In addition, in the absence of information on the affects of surface sterilization on germination, we also used a standard procedure (Guiragossian-Kiss and Kiss, 1998).

With the exception of the moisture level experiment, spores were surface sterilized in a 7% (v/v) commercial bleach solution with 0.1% (v/v) Triton X-100 for 20 min. Spores were then rinsed in sterile ddH_2O, sown on a modified Knudson's-C medium (C-Fern, 2001; 3.7 mM $(NH_4)_2SO_4$, 4.2 mM $Ca(NO_3)2{\cdot}4$ H_2O, 1.8 mM KH_2PO_4, 27.0 μM $FeSO_4{\cdot}7H_2O$, 17.5 mM sucrose, 10 μM H_3BO_3, 10 μM $MnSO_4{\cdot}H_2O$, 3 μM $ZNSO_4{\cdot}7H_2O$, 0.1 μM $Na_2MoO_4{\cdot}2H_2O$, 0.01 μM $CuSO_4{\cdot}5H_2O$, 0.01 μM $CoCl_2{\cdot}6H_2O$) with 1.2% (w/v) sucrose (35 mM) in 9-cm Petri dishes (Guiragossian-Kiss and Kiss, 1998). All plates were prepared and poured from the same Knudson's-C preparation to ensure a consistent concentration of sucrose. Dark control plates were wrapped in aluminum foil and incubated in the same conditions as other plates within the same experiment. Spores were incubated for 7 d under various experimental conditions, with the exception of the moisture experiment, during which spores were treated for 10 days. When feasible, conditions for each test (Table 1) were maintained at pH 5.5, 25°C, 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of white light, and saturated (agar medium). However, the parameters of some experiments required different conditions. For example, with respect to light quality, spores were incubated at 33°C and at 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to achieve maximum and consistent light intensity in each light quality.

VARIABLES: pH, TEMPERATURE, LIGHT INTENSITY, AND LIGHT QUALITY.—To examine the effects of pH on spore germination, spores were sown on Knudson's-C of pH 4.5, 5.5, 6.5, and 8.5. Knudson's-C does not buffer well at pH 7.5 and this pH was not used. To test the effects of temperature on spore germination, spores were incubated at 4°C, 25°C, and 33°C. Light intensity was established by using either GE Halogen Ultra PAR 38 (90 watts) or Sylvania Halogen XTRA PAR 38 (120 watts) and by varying the distances between the light source and the spores. Light intensity was verified with a Li-Cor Quantum/Radiometer/Photometer, model LI-185. Light intensities were: 0 (dark), 10, 50, 75, 100, 125,

150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Treatments were contained within chambers to avoid incidental light and cooled with an electric fan system. Dark treatments were prepared by wrapping Petri dishes in foil after they were inoculated and sealed with Parafilm. Light qualities tested were blue, red, white, green, far red, and dark. These light qualities were established using plexiglass filters (Cadillac Plastics, Southfield, MI). Wavelengths for light filters were measured using Data Logger Pro software and were: white (420–710 nm), blue (420–570 nm), green (500–595 nm), red (600–695 nm), far red (650–705 nm).

DARK CONTROL (DARK PLUS VS. TRUE DARK).—During the surface sterilization and sowing process, spores are typically exposed to white light for approximately 45 min and this characterizes spore preparation for all experiments. Even dark treatments receive this white light prestimulus (Dark Plus). To test the affects of this white pre-stimulus, the germination rate in the standard dark control preparation (Dark Plus) was compared with the germination rate of spores that were surface sterilized and sown in 0.04 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of white light (True Dark).

MOISTURE LEVELS.—To avoid introduction of additional moisture, spores were not surface sterilized and were sown on sterilized filter paper (Whatman #1, 9 cm) that were wetted with Knudson's-C (no sucrose to avoid contamination that would hinder germination, no agar). Moisture levels were: 0, 10, 20, 30, 40, and 50 $\mu l \cdot cm^{-2}$. Germination rates were counted at 7 d, but protonema were allowed to develop to the tenth day for observation and measurement.

DATA ANALYSIS AND SAMPLING.—To ensure that spores, which germinate quickly, were counted within only a few hours of each other, but to also obtain large sampling, experiments were conducted independently. Testing all variables at one time would sacrifice the integrity of the counts. Spores were sown on 4–10 plates per treatment, depending on the parameters of the experiment. For example, only 4 plates were used in light quality experiments to ensure that all plates were placed within the center of the filters and received the same light intensity. Spores were scored as germinated or non-germinated on a haphazard basis up to 300 spores per plate, depending the number of plates allowed by the parameters of the experiment ($n \approx 400–1200$ spores per treatment). Spore germination was counted when the exine had ruptured and protonemal cell extrusion was visible. Because germination/non-germination is a binomial score, the data were transformed using the arc sine transformation method to ensure normality. An analysis of variance ($\alpha = 0.05$) was then performed to determine significance of differences in results. For clarity and continuity, the transformed means and standard deviations were used in figures.

POROSITY, SPECIFIC RETENTION, AND ACTUAL RETENTION.—Rock samples were obtained with special permission from Reis Biological Station (RB), Steeleville, MO, from bluffs along Big River at Mammoth Road (MR), approximately 1.5 mi south of MO Highway H, and from private land in Cedar Hill (CH), MO approximately 1.5 east of Highway 30 along Highway BB. Because collection was destructive, rock sample sets were purposely limited to 10 approximately 2.5 cm^3 pieces. Two sample sets were collected from each site. One set was

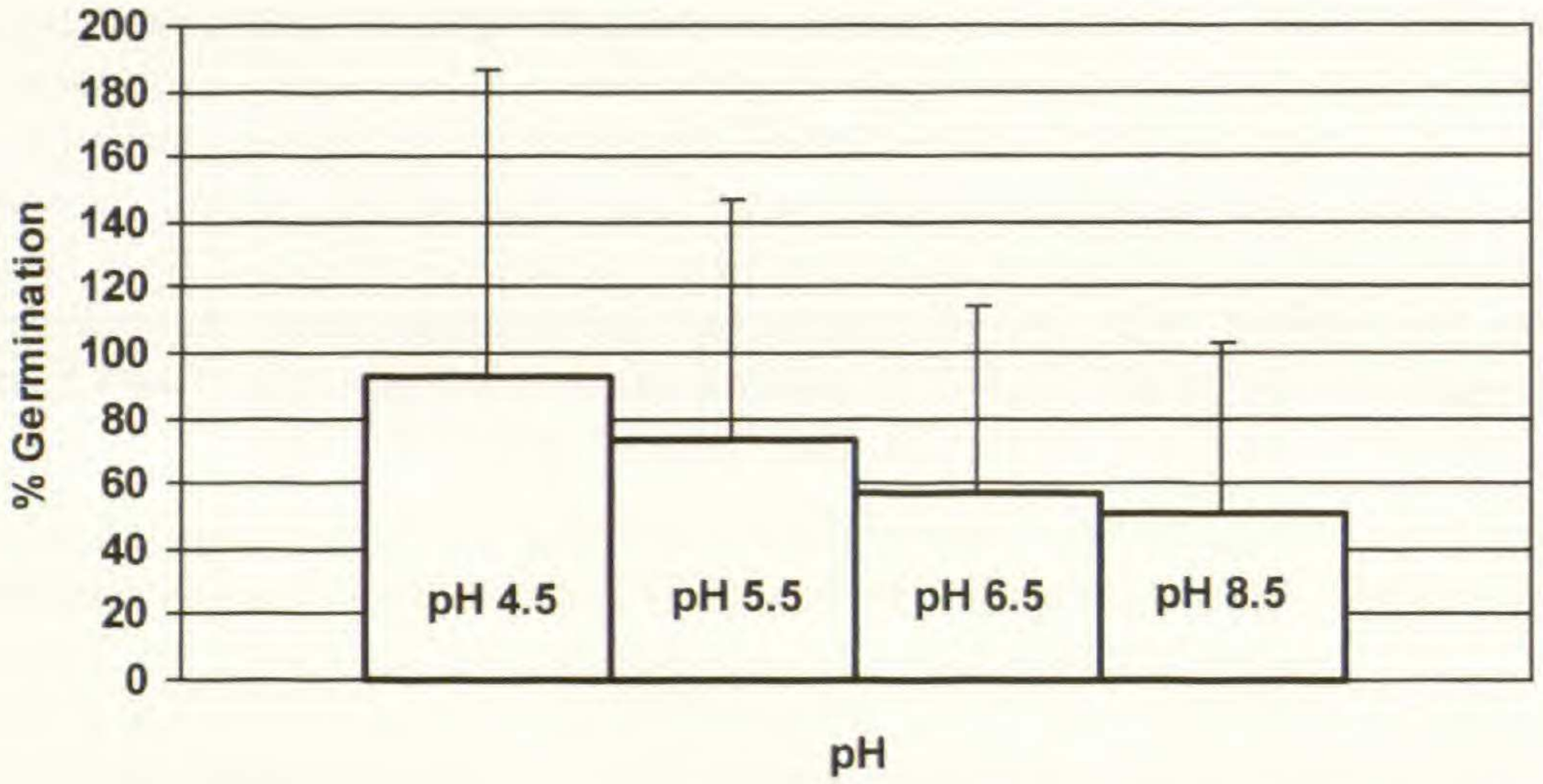

FIG. 1. Effects of pH on spore germination, transformed data. *Cheilanthes feei* germinated best in pH 4.5. There was no difference between pH 6.5 and 8.5. Dark germination rates (not shown) were less than 5%.

collected from *C. feei* habitat and one set was collected from the same stratum, but elsewhere on the bluff where *C. feei* did not inhabit. Porosity was determined as $n = 100[1-(P_b/P_d)]$, where P_b (bulk density) is defined as the original sample oven dried weight (g) divided by the saturated pre-oven dried volume (cm^3) and P_d (particle density) is defined as the original sample oven dried weight (g) divided by the mineral matter volume (cm^3). Specific retention of the substrate was determined as the amount that the substrate can retain against gravity divided by the total volume (Fetter, 1988). Actual retention values were subsequently determined for cm^2 planes within the substrate. This was calculated as the amount of water (cm^3) retained against gravity divided by the pore space available for water retention and further divided by 10 for comparison with laboratory conditions.

RESULTS

VARIABLES: pH, TEMPERATURE, LIGHT INTENSITY, AND LIGHT QUALITY.—Germination occurred in a broad pH range (Fig. 1). *Cheilanthes feei* spores (n = 400–1200) germinated at the highest rate in acidic pH (pH 4.5 and 5.5). Limestone pH varies, but is basic (*ca.* pH 8.3; M. Aide, pers. comm.). pH 7.5 was not tested, since Knudson's-C does not buffer well at this pH. Dark controls (Dark Plus; not shown) were 5% or less in all pH. *Cheilanthes feei* (n = 800–1000) spores germinated optimally at 25°C (Fig. 2). Note that germination also occurs at 33°C and at 4°C. Dark controls (Dark Plus) are not shown. Dark germination rates at 25°C and 33°C were less than 5%. At 4°C in the dark (storage conditions), no germination occurs. Spores (n = 600–1200) germinated under a wide range of light intensities (Fig. 3). All germination rates are low but comparable to expected values at 33°C (25°C is difficult to maintain under the stronger light intensities). The optimal germination rate occurred at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and there were significant differences between the highest germination rate (100

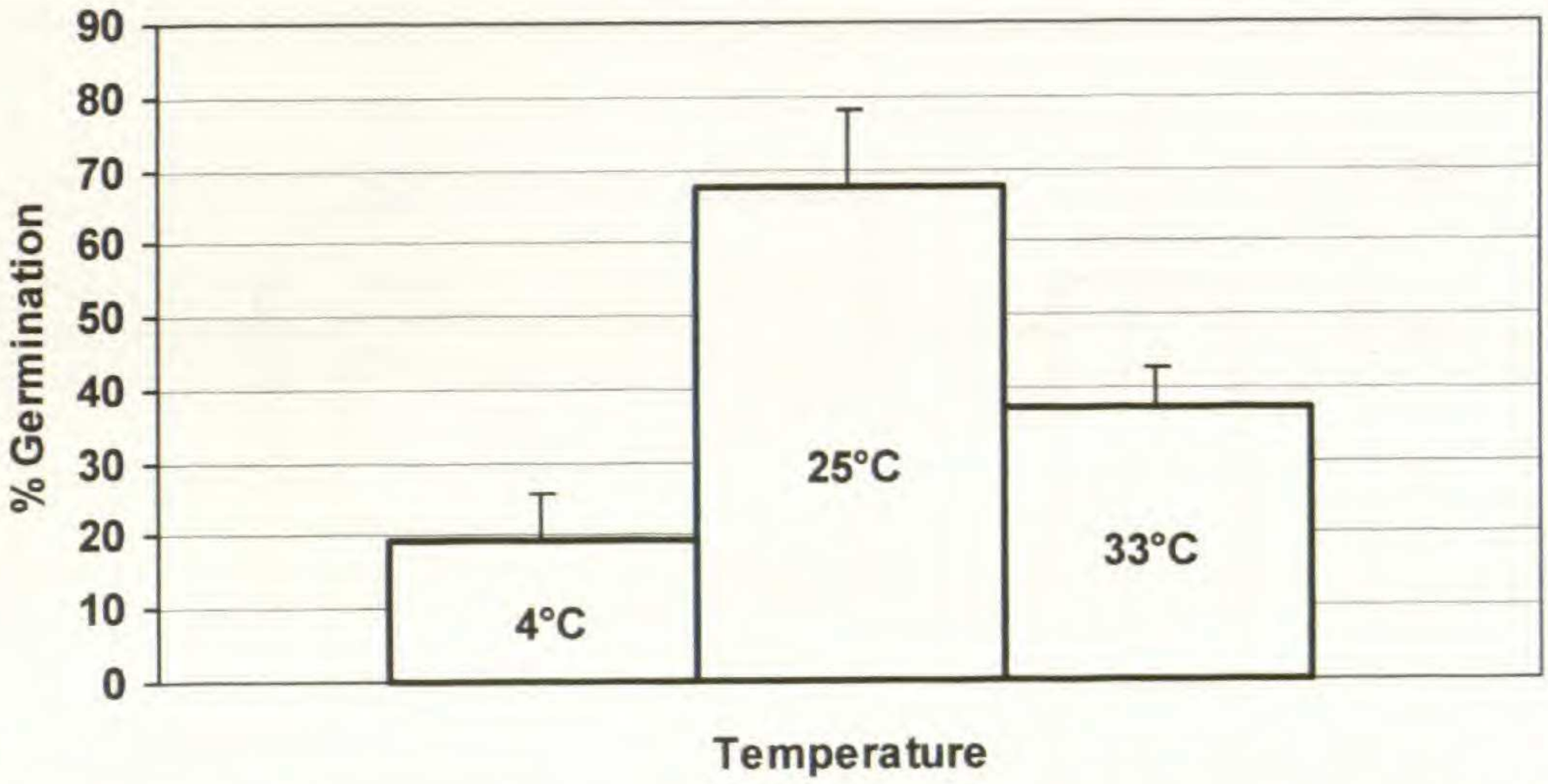

FIG. 2. Effects of temperature on spore germination, transformed data. The optimal temperature for spore germination is 25°C. Germination in 33°C and 4°C is significantly less. However, spores germinated in all temperatures. In the dark controls (not shown), no germination occurred at 4°C (storage conditions). In the dark, *C. feei* germinated below 5% at 25°C and 33°C.

$\mu mol \cdot m^{-2} \cdot s^{-1}$), other light intensities, and the dark control (Dark Plus) rate. *Cheilanthes feei* spores germinated under all light qualities, even in the Dark Plus controls (Fig. 4). Germination rates in different light qualities varied greatly, but not significantly. For example, there was no significant difference between white, far red and green. A notable difference was in the far-red treatment in germinated spores. All germinated spores in this treatment were at the 2-cell protonemal stage when scored. This was not observed in any other light quality treatment. Dark controls (Dark Plus) germinated at 7.7%. Preparation methods markedly affected germination rates (Fig. 5). Without the 45 min white light prestimulus (True Dark), germination occurred at levels that

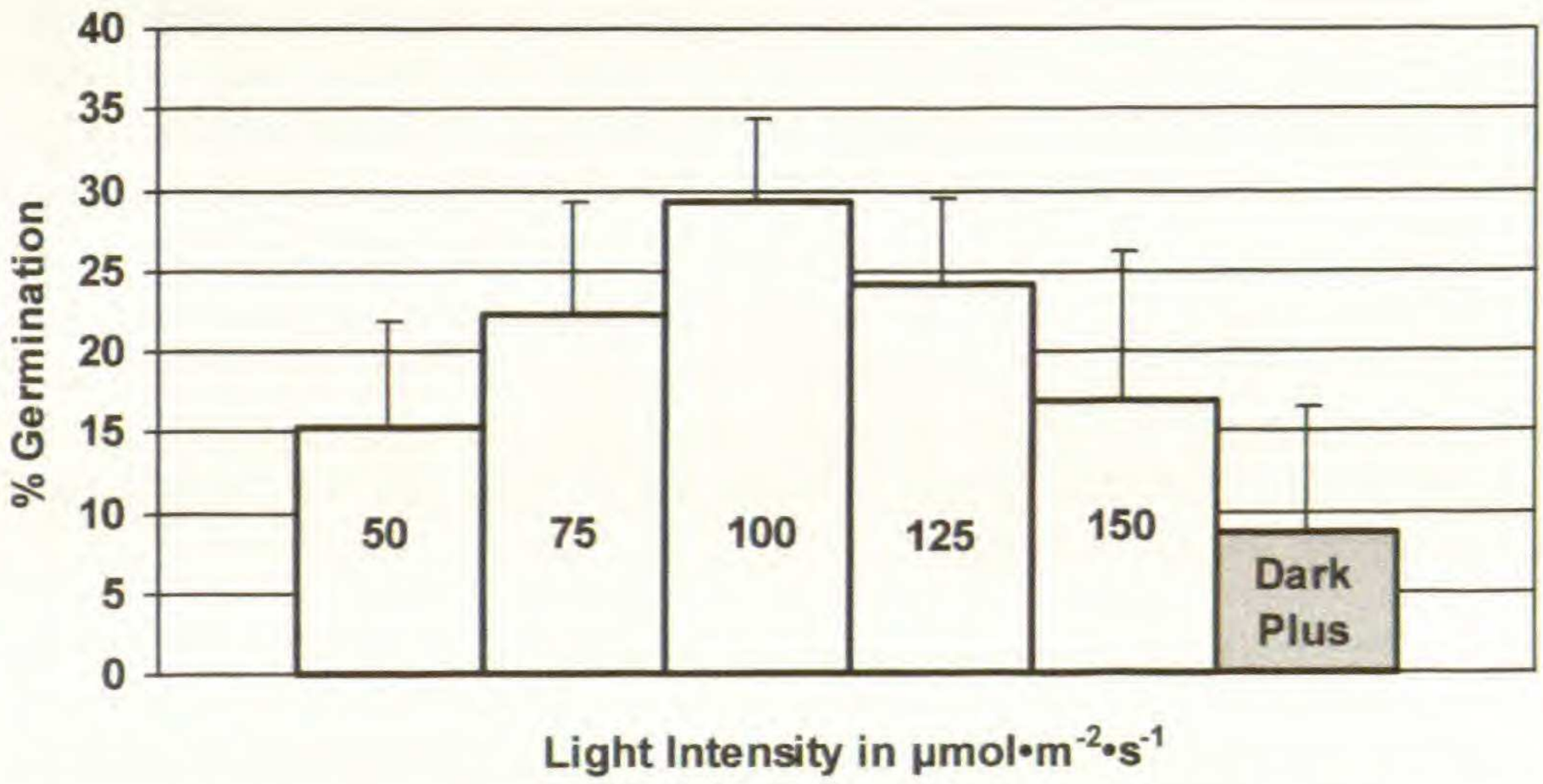

FIG. 3. Effects of light intensity on spore germination, transformed data. Germination rates for were predictably low in 33°C. The optimum rate was under 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and there were significant differences between light treatments and the dark controls.

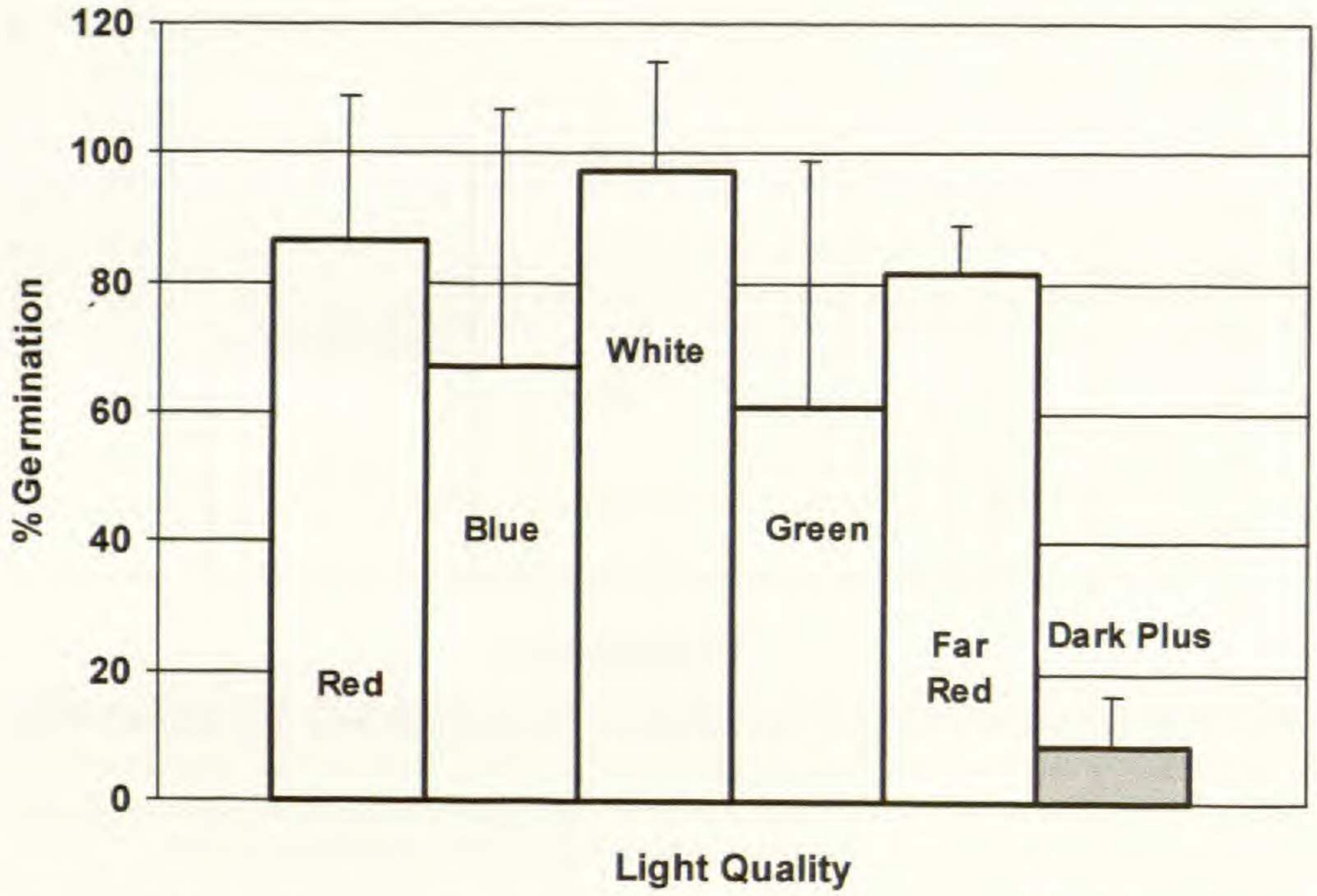

FIG. 4. Effects of light quality on *C. feei* spore germination, transformed data. With the exception of far red, results in each light quality were highly variable. No significant difference exists between germination rates in any light quality. However, spores germinated in far red light were found at the 2-cell protonemal stage. This did not occur in any other light quality treatment. Dark germination was at 7.7%.

exceeded any previous light treatments. Dark Plus: n = 590; True Dark: n = 4300.

MOISTURE LEVELS.—Although *Cheilanthes feei* spores germinated in the dark on dry filter paper, germination rates were optimized in the light at 20–50 $\mu l \cdot cm^{-2}$ (Fig. 6). There was no significant difference between these light treatments, but there was a significant decrease in germination in the dark controls between 20–50 $\mu l \cdot cm^{-2}$ as moisture increased. In addition, there was a substantial difference in protonematal presence and maturity. For reference, 20 $\mu l \cdot cm^{-2}$ will support mildew growth and is merely damp to the touch. Microscopically, no liquid stands between the fibers of the filter paper. At 30 $\mu l \cdot cm^{-2}$, a film of water coats the fibers. Above 40 $\mu l \cdot cm^{-2}$, the filter paper is saturated and water stands between the fibers. Protonema that germinated in 20 $\mu l \cdot cm^{-2}$ were approximately 200 μm in length when scored and exhibited planar growth, but protonema in 40 and 50 $\mu l \cdot cm^{-2}$ were only 100 μm in length and still filamentous (Fig. 7).

POROSITY, SPECIFIC RETENTION, AND ACTUAL RETENTION.—Reis Biological Station (RB) is farthest from the St. Louis, MO metropolitan area (approximately 66 mi) and is a protected area for biological studies. Mammoth Road (MR) is a rural site (approximately 30 mi from the St. Louis metropolitan area) that overlooks a boat ramp and fishing area. The area is worn by foot traffic. Cedar Hill (CH) is private rural land that is approximately 16 mi outside of the

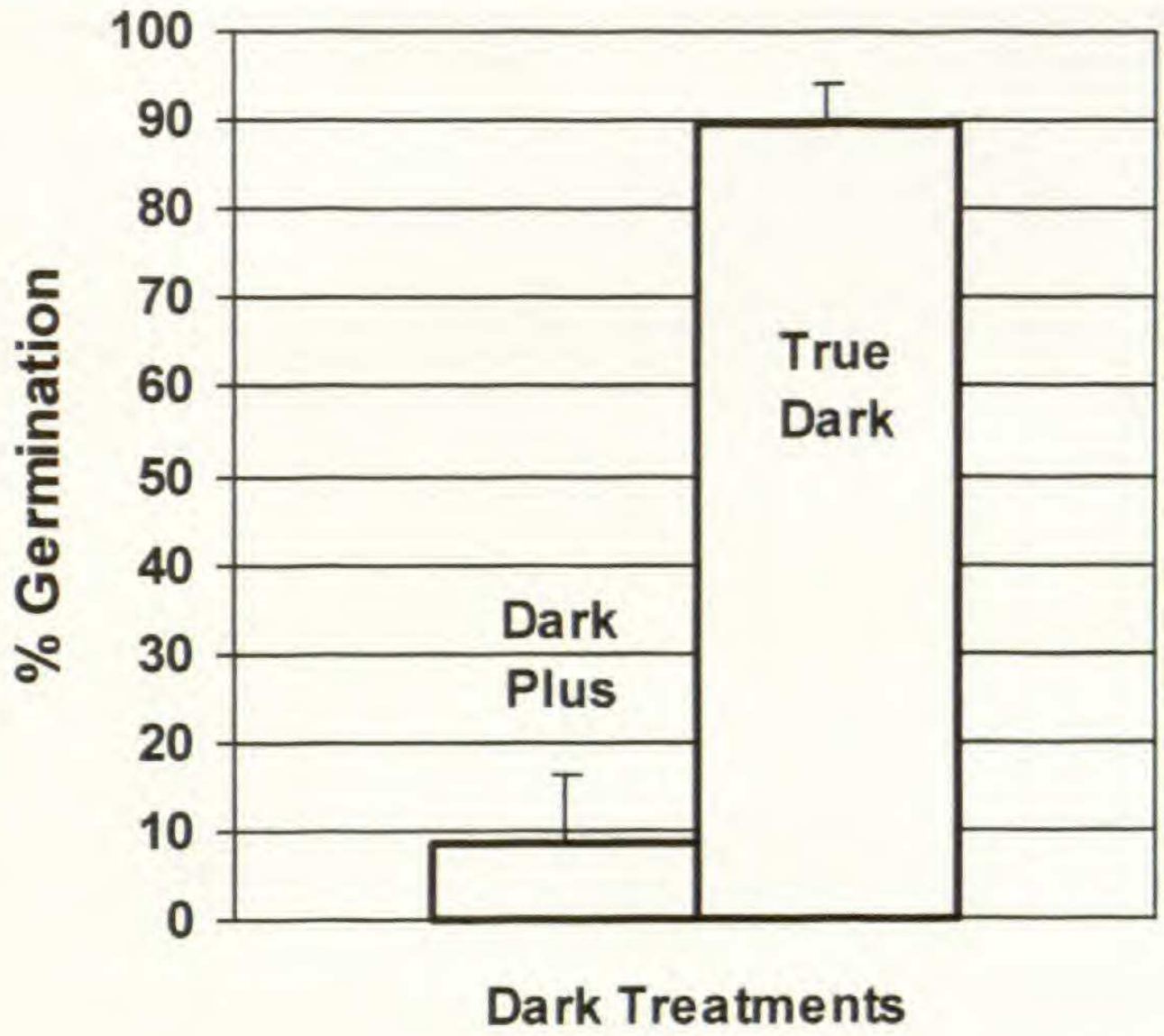

FIG. 5. Effects of white light prestimulus on dark germination in *C. feei*, transformed data. The 45 min white light prestimulus (Dark Plus) strongly affects the germination rates of dark germinated spores. *Cheilanthes feei* germination in True Dark scores between 80–100%.

metropolitan area and the closest of the sites to the city (St. Louis, MO metropolitan area).

Average porosity and specific retention increased with distance from the city. Porosity is the amount of pore space in a rock sample compared to the total volume, and is expressed as a percentage (Fig. 8A). Porosity means

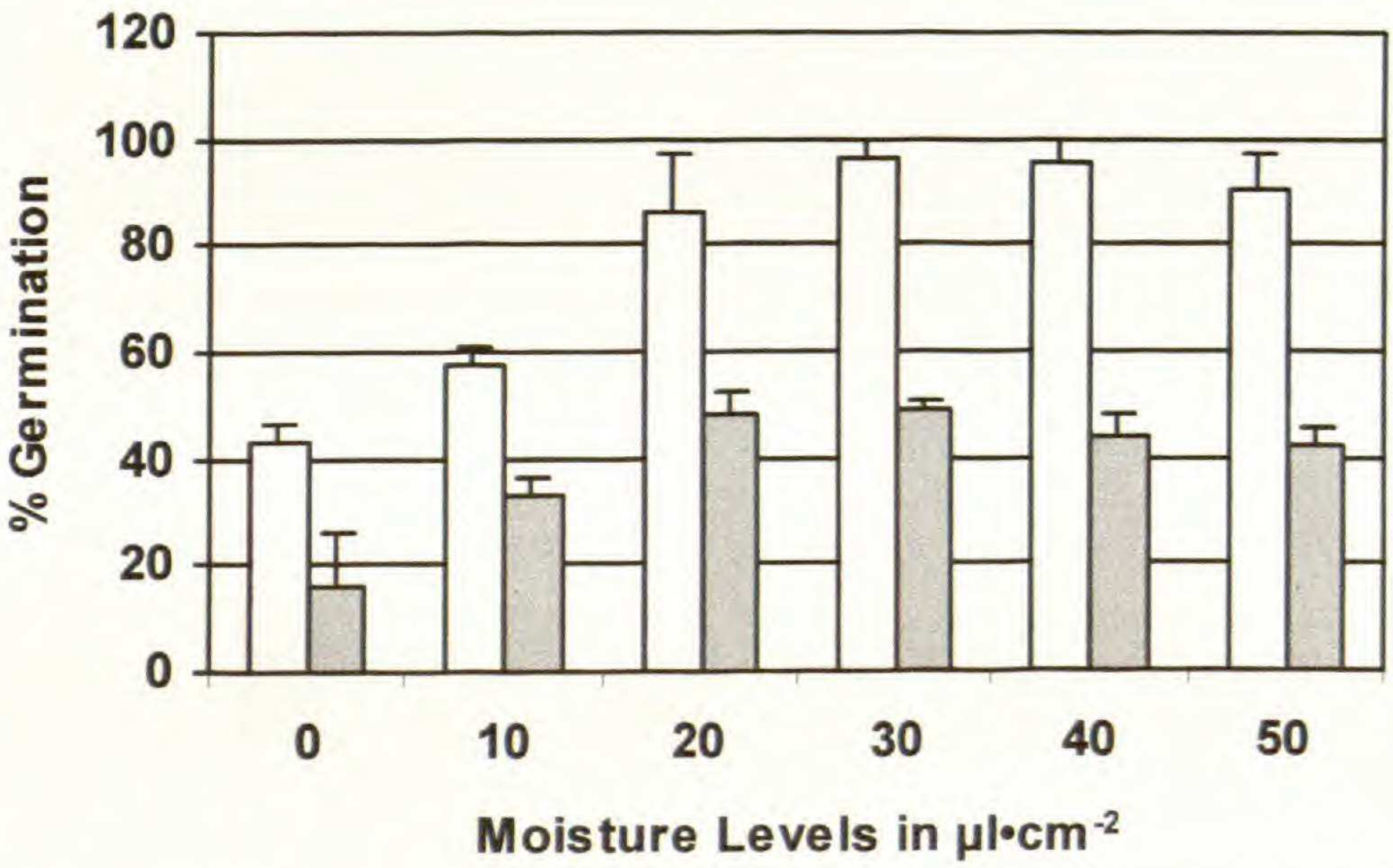

FIG. 6. Effects of moisture levels on *C. feei* spore germination, transformed data. In the light (white), germination optimizes at 20–50 $\mu l \cdot cm^{-2}$. There is no significant difference between germination rates in these treatments. Dark germination (Dark Plus; gray) at 20–50 $\mu l \cdot cm^{-2}$ begins to decrease gradually and there is a significant difference between 20–50 $\mu l \cdot cm^{-2}$. Note that germination occurs on dry filter paper in the dark.

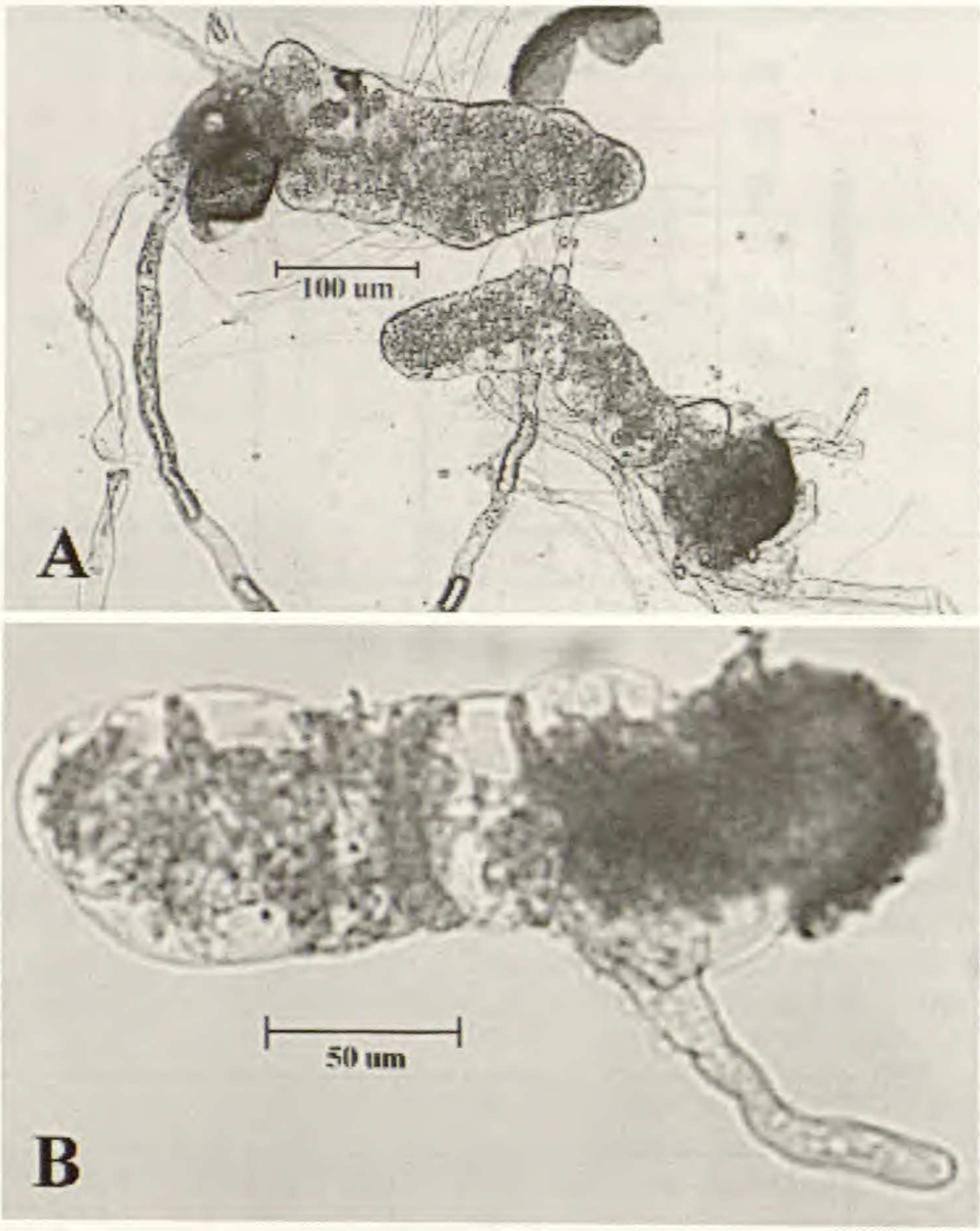

FIG. 7. Protonematal maturity at different moisture levels. (A). Protonemata grown at 20 $\mu l \cdot cm^{-2}$ are approximately 200 μm in length and exhibit planar growth. (B). Protonemata grown in 50 $\mu l \cdot cm^{-2}$ are approximately 100 μm in length and are filamentous.

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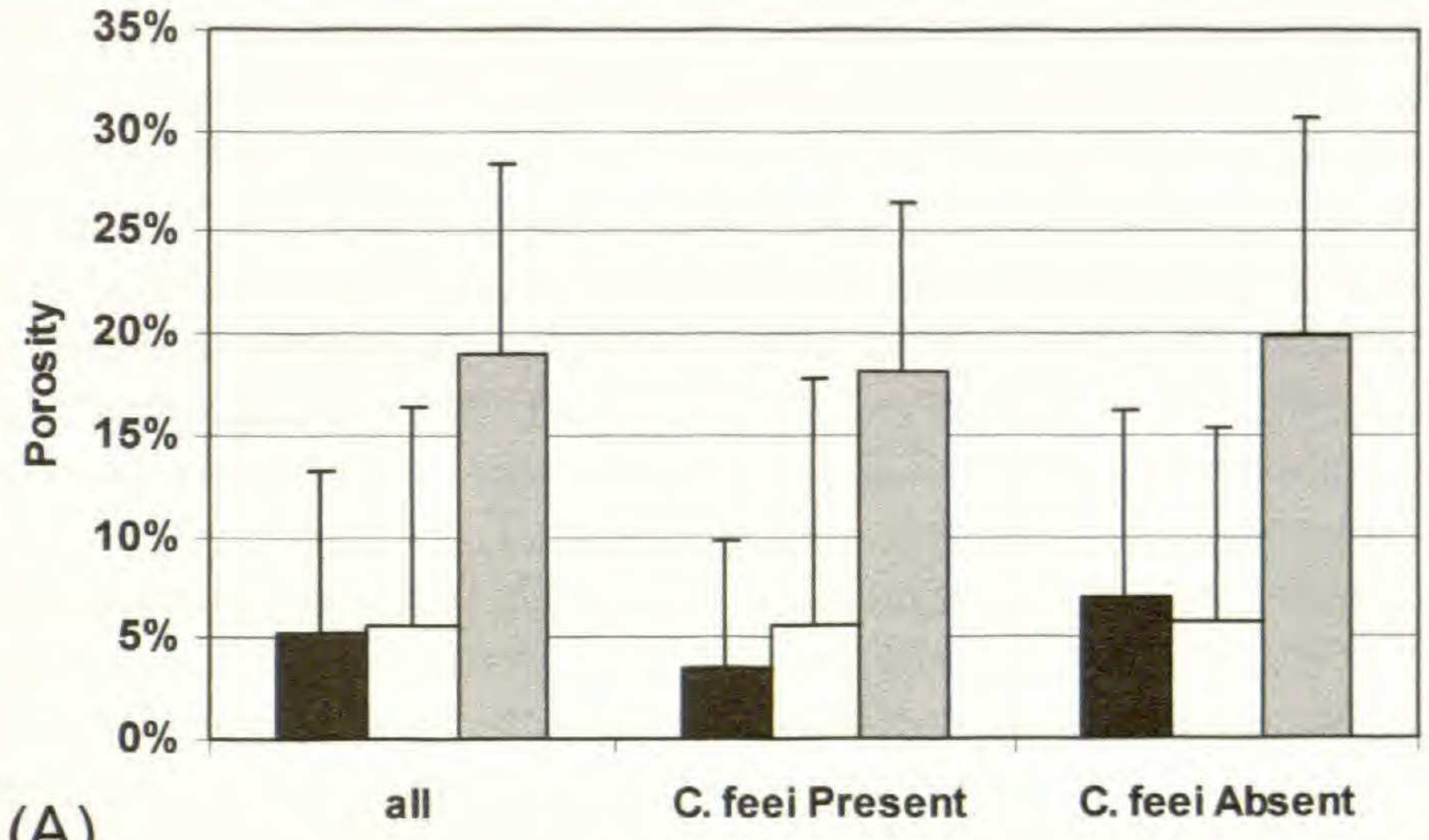
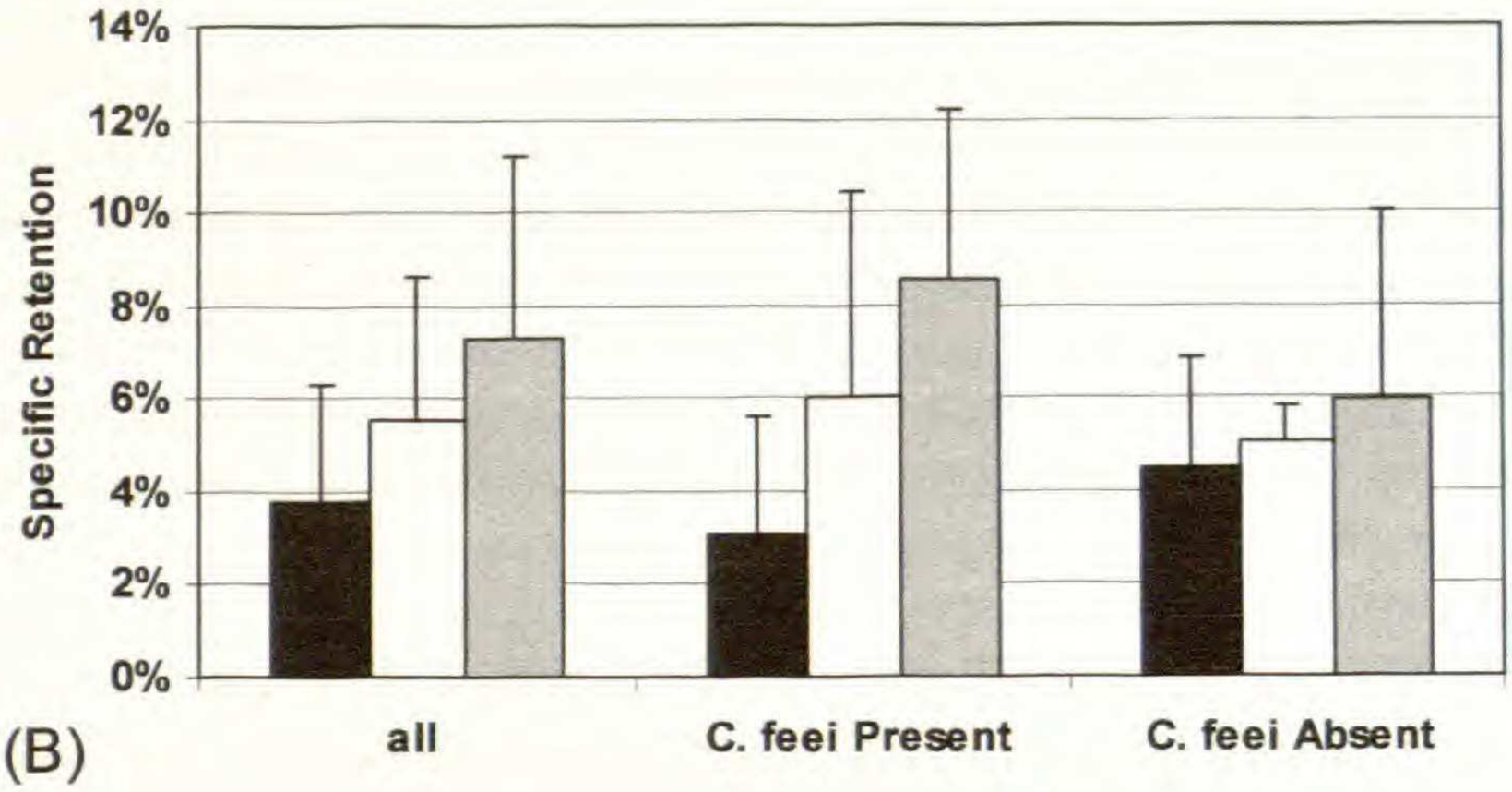
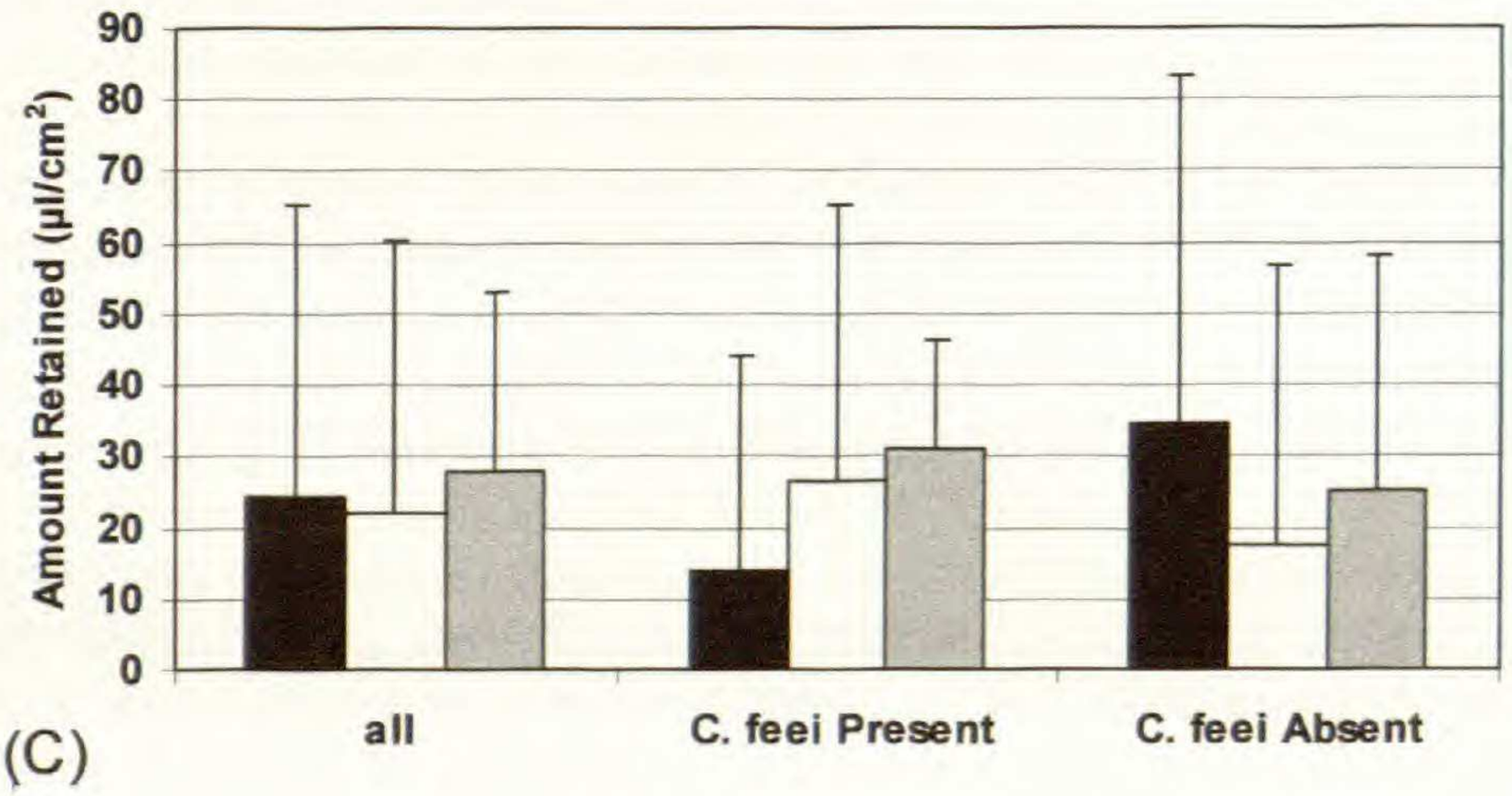
FIG. 8. (A). Mean porosities at Cedar Hill (black), Mammoth Road (white), and Reis Biological Station (gray). There was no significant difference between areas within the same stratum that contained *Cheilanthes feei* and areas in which *C. feei* was absent. (B). Specific retention increased with porosity and with distance from the metropolitan area. Conversely, variation in specific retention decreases with distance. (C). Actual amount retained in $\mu l \cdot cm^{-2}$ was calculated as the specifically retained water distributed equally throughout the pore space and mathematically expressed within one plane. Means for all rock samples taken from each site fell between 20–30 $\mu l \cdot cm^{-2}$.


35%
30%
25%
20%
15%
10%
5%
0%
Porosity
all
C. feei Present
C. feei Absent

(A)


14%
12%
10%
8%
6%
4%
2%
0%
Specific Retention
all
C. feei Present
C. feei Absent

(B)


90
80
70
60
50
40
30
20
10
0
Amount Retained (µl/cm²)
all
C. feei Present
C. feei Absent

(C)

increased with distance from the St. Louis metropolitan area. There was a significant difference between mean porosity at Reis Biological Station and that at Cedar Hill. However, there was no significant difference between Mammoth Road porosities and porosities measured from samples at Reis Biological Station and Cedar Hill. In addition, there were no differences in the porosities between the areas that contained *C. feei* and those that did not. Still, there was substantial variation in the data, so that the standard deviations neared or exceeded the mean. This variation was consistently smaller at RB, a protected site.

Specific retention values, amounts of water retained against gravity as a percentage of the total volume of the sample, mirrored results for porosity (Fig 8B). There was no significant difference between MR and RB or CH, but a significant difference between CH and RB. Variation in samples was also large and there were no significant differences between specific retentions measured in samples taken from where *C. feei* was present and where it was absent.

Finally, there were no significant differences between actual amounts retained (Fig. 8C). This applied to comparisons between sites and to comparisons between samples taken from where *C. feei* was present and where it was absent. Actual amounts retained were determined by the amount of water held against gravity divided by actual pore space. These means fell between 20–30 $\mu l \cdot cm^{-2}$. The few exceptions, such as at Cedar Hill where *C. feei* was present, did not vary significantly from the mean. Overall, the average actual retentions (not shown) for all sites was 24.8 $\mu l \cdot cm^{-2}$. The average actual retention of samples taken from where *C. feei* was present was 23.9 $\mu l \cdot cm^{-2}$ and 25.7 $\mu l \cdot cm^{-2}$ from where it was absent.

Discussion

Parameters for *Cheilanthes feei* Distribution.—Based on spore germination requirements, *Cheilanthes feei* has the potential to occupy a broad array of environments. There is no particular restriction to any one condition and germination itself is highly variable. These data suggest that *C. feei* is extremely versatile. First, substrate pH is nonrestrictive. *Cheilanthes feei* spores germinated in each pH range tested (Fig. 1). Slightly acidic pH promoted slightly better germination rates than at basic pH. Another variable, temperature, also failed to substantially affect germination (Fig. 2). Admittedly, higher temperature, 33°C, inhibited spore germination, but over 35% of the spores still germinated. Furthermore, spores germinated in the cold (under continuous white fluorescent light), although the germination rate was markedly less than at 25°C. Still, *C. feei* spores do not germinate in the dark at 4°C. A rise in temperature appears to be important in germination, from dark, cold storage to warmer conditions, but the basis for this is unclear. A rise in temperature may promote the expression of hormones prior to germination or the addition of light could increase sensitivity to hormones present (Davies, 1995). Membrane integrity in the cold may also be compromised and inhibit germination (Cuming, 1999), but spores maintain long term viability in storage

and cold leakage is an unlikely issue. Third, light intensity and light quality made little difference overall in spore germination. Optimal conditions for light intensity and light quality are evident. In continuous white light, 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, they germinate well (Fig. 3), but spores still germinate in all light intensities and in dark with a white light prestimulus (dark plus). The optimal condition for light quality is actually in darkness (Fig. 4–5), with no light prestimulus (true dark). Mean rates for germination in True Dark (Fig. 5) treatments were no higher than in white light, but the variation in samples was notably reduced. Finally, *C. feei* spores "imbibe" and germinate with no visible source of moisture available and in moist or saturated conditions. Clearly, germination is best when the substrate is moistened or saturated (Fig. 6). However, fern spore germination on dry medium is noteworthy. Although negligible, relative humidity and/or unobservable moisture present on the exine were potential sources of moisture. These spores apparently possess the capacity to uptake water for germination with only high relative humidity as a moisture source. Still, these conditions are present within storage, but imbibition does not occur. Once again, a temperature rise is likely required prior to germination. This is followed by imbibition and germination, optimally following burial beneath debris in limestone crevices.

Taken as a whole, these data on spore germination rates in various conditions indicate that *Cheilanthes feei* spores are neither bound by the inability to compete (with regard to germination) in alternative habitats, nor by the inability to survive in mesic habitats due to morphological or physiological adaptations, nor by a requirement for optimal growth conditions. They can germinate under a wide range of conditions and only require a rise in temperature. Although these spores exhibit nearly 100% germination in certain conditions, they germinate adequately under most conditions. Therefore, there is little with respect to spore germination that explains the narrow niche of this fern, only its broad distribution.

RESTRICTION OF *CHEILANTHES FEEI* TO ITS NARROW NICHE.—One remaining explanation for the narrow niche of *Cheilanthes feei* in southeast Missouri lies in habitat specificity due to substrate moisture level and protonemal moisture requirements. *Cheilanthes feei* spores can germinate in most moisture levels and do well in saturated conditions, but protonema do poorly in saturated conditions. There is, then, a narrow range of conditions in which *C. feei* can germinate and development optimally. *Cheilanthes feei* spores germinated optimally (80–100%; Figs. 1–5) without a light stimulus, at 25°C and pH 5.5, and when moisture levels were between 20–50 $\mu l \cdot cm^{-2}$ (Fig. 5). Although spores germinated well between 20–50 $\mu l \cdot cm^{-2}$, data from the moisture level experiment on viability of germinated spores and protonema reveal that protonema develop farther in lower moisture levels (20–30 $\mu l \cdot m^{-2}$; a film of water coats the substrate fibers) than protonema in cultures with higher moisture levels (40–50 $\mu l \cdot m^{-2}$; water stands between substrate fibers). Protonema in lower moisture levels were 200% larger than those in greater

moisture levels (Fig. 7). This may be the result of a disparity in germination time or in protonemal vigor.

SUMMARY OF GROWTH REQUIREMENTS.—Optimal conditions for *C. feei* spore germination and subsequent protonemal development may be summarized as shade or complete burial, moderate temperature, in any pH, but with only 20–30 $\mu l \cdot m^{-2}$ throughout the germination and protonemal stages. The first three requirements are broad and can be fulfilled in many habitats. The latter is the more difficult to secure and is the restricting factor.

POROSITY AND MOISTURE RETENTION ARE RESTRICTING FACTORS.—Based on moisture requirements, *Cheilanthes feei* can only occupy environment types that offer a narrow margin of moisture conditions for germinated spores and growing protonema (20–30 $\mu l \cdot m^{-2}$). Sedimentary substrates offer a consistent amount of moisture and air space. The amount of moisture retained depends on porosity and specific retention. Porosity is defined as the percentage of sedimentary rock that is actually pore space. The primary determiner of porosity is weathering. Weathering can be induced chemically from reactions within the rock components or from reactions between rock components and pollution. Weathering can also be induced mechanically by wind, rain, ice, etc. Within the Emminence-Potosi Dolomite formation in southeast Missouri, mean porosity (Fig. 8A) increased and variability, which was substantial, decreased with distance from a metropolitan area (St. Louis, MO). Specific retention, the amount of water retained against gravity and expressed as a percentage of the total rock volume, also increased with distance (Fig. 8B). However, variation decreased slightly with distance from the city. Given that the chemical composition of the substrate is relatively consistent, the amount of weathering, possibly pollution-induced chemical weathering, altered the porosity and specific retention. The important consideration for *C. feei*, however, is not necessarily the porosity or specific retention, but the amount that the rock substrates actually retain *within* the available pore space. Pore space, concretion, and subsequent blockage of pores are unique for each site during the weathering process, so that distance from a pollutant source would affect the degree of porosity, specific retention, and variability between samples, but result in a mean actual retention that is or is not adequate to support *C. feei* colonization. In samples taken from *C. feei* habitat, the actual amount of water retained was mathematically distributed throughout the entire pore space and rendered within one plane as $\mu l \cdot cm^{-2}$. The means for all three sites ranged between 20–30 $\mu l \cdot cm^{-2}$ (Fig. 8C). The actual retained amounts were achieved by a wide range of porosities and therefore, degree of weathering. With few exceptions, actual retention means from *C. feei* collection sites fell within this narrow margin. The exceptions varied from the means with no significant differences. These data suggest that, within *C. feei* habitat in southeast Missouri, moisture level requirements, which restrict *C. feei* to a narrow niche, are satisfied by and are subject to porosity of its limestone substrate. Future studies are needed to determine if these is consistent with alternative substrates in other North American *C. feei* habitat.

IMPLICATIONS FOR THE FUTURE OF *CHEILANTHES FEEI*.—*Cheilanthes feei* is slow to establish or re-establish after road cuts and mining. Based on data taken from this study, *C. feei* habitat in southeast Missouri is non-renewable. Mechanical weathering is a long-term process and substrate characteristics cannot be readily mimicked or replaced. Chemical weathering is more rapid. Chemical weathering induced by pollution may open up new *C. feei* habitat. Unfortunately, chemical weathering may simultaneously destroy existing habitat. Therefore, additional studies on formations across the western United States, southwestern Canada and north central Mexico are imperative to determine whether *C. feei* habitat in North America is at risk.

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